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Developments in cryo-crystallography : Application of new techniques to BPTI and Concanavalin A.

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Dramatic improvements in data quality for macromolecular crystals can be attained by cryogenic data collection. The advantages are manifold: problems are alleviated in almost all stages of the structure determination process. Firstly, the detrimental effects of radiation damage are postponed or eliminated, so that complete data sets can be collected from just one crystal, eliminating errors introduced by inter-crystal merging and scaling. Secondly, reduced thermal motion effectively increases the contrast in electron density maps, which facilitates interpretation. No radiation damage, and reduced thermal smearing of electron density, combined with possible structural re-arrangements to more ordered states can lead to substantially higher limiting resolutions which do not deteriorate with time. We have recently completed low-temperature structural investigations of BPTI and concanavalin A.

In BPTI, flash cooling to 125 K induced small structural changes in the molecule which effectively locked in a single conformation for the two carboxyl terminal residues that had previously defied location in work at room temperature. In addition, the magnitude of disorder in side chains was reduced, and over 90% of all water was visible in electron density maps.

Cooled crystals of native concanavalin A invariably diffract x-rays to at least 1.2 Å, and often beyond 1.0 Å. Data extending to 1.2 Å resolution have confirmed recent reassignments of several amino acids in the primary sequence and have revealed readily interpretable electron density in previously untraceable loop regions. The solvent region in these crystals is also considerably more ordered at low temperature.

In concanavalin A, a non-reversible, non-destructive phase transition lengthens the *b* and *c* axes disproportionately with respect to the *a* axis on raising the temperature to between 160 K and 165 K. Investigations into the nature of this phase transition is ongoing.

In our work on cryocrystallographic techniques we have developed new implements for crystal handling and storage. We often cool crystals immediately after mounting, by dipping them into liquid N_2. Contrary to popular belief, this results in very rapid cooling. For transfer to a diffractometer the submerged mounting pin with crystal is inserted into a special holder of novel design that keeps the crystal cold during transfer to a running cold stream on a diffractometer. A reverse operation allows the safe removal of the crystal, which can then be stored in a standard cryo-refrigerator for later use.

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